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STUDIES ON THE BIOLOGY OF THE DAMSELFLY *ISCHNURA VERTICALIS* SAY, WITH NOTES ON CERTAIN PARASITES

BY EVELYN GEORGE GRIEVE
ITHACA, N. Y.

INTRODUCTION

Although *Ischnura verticalis* is a common and widely distributed damselfly its biology has been little studied. In the present investigation more than one hundred nymphs were hatched from eggs laid in the laboratory, and each was reared in a separate container to make possible individual life history records. In conjunction with the rearing experiments, observations were made on oviposition, hatching, growth, molting and transformation, and on female polychromatism. There was discovered a disease, due to the presence of certain green protozoa in the rectum of the nymphs, that was associated with their high mortality. This led to a preliminary survey for parasites; and the cercariae of two Distome Trematodes were found in many of the nymphs and adults, during part of the season. All these matters are discussed in the following pages.

THE IMMATURE STAGES

Rearing work was begun with the full-grown nymphs which were collected early in the spring of 1931 from one of the trout ponds of the Cornell Experimental Fish Hatchery, at Ithaca, N. Y. From the first generation, which were the stock adults, eggs were obtained for the life history studies.

The females will oviposit in various soft-stemmed aquatic plants. The most convenient for my purpose was a round-stemmed spike rush, *Eleocharis palustris*, which was kept growing in the screen-covered tanks. These stems are flexible, and one or two could be bent into the water each day, and held down by the surface film. The females would oviposit only in these floating stems, and each day's eggs could be conveniently removed and dated. In Fig. 1 E is shown a longitudinal section of a stem, with the eggs embedded in the plant tissue.

The Egg and the Sperm.—Ripe spermatozoa were taken from the spermatheca of a mature female. Head and nucleus could not be clearly distinguished although the anterior third was blacker than the remainder of the sperm. The width is fairly uniform, though tapering slightly posteriorly; and the whole body of the sperm has an undulating, possibly spiral, form. It terminates in a short forked tail. The length, exclusive of the forked tail, averages about 0.04 mm., and the forks of the tail, approximately 0.007 mm.

The eggs of this species are elliptical, slightly curved, the head end being a little enlarged and surmounted by an operculum or cap. The eggs are quite uniform in size, averaging 0.83 mm. long, and tapering in width from 0.18 mm. near the anterior end to 0.12 mm. near the posterior end.

The length of the egg stage is quite uniform within any one lot of eggs, each lot consisting of all those collected from the oviposition tank in one day. It varies from twelve to twenty days, dependent apparently on temperature, with ratios as shown in the accompanying table.

	Duration of egg stage									
No. of days	12	13	14	15	16	17	18	19	20	
No. of lots	1½	4½	3	2	1	1	1	1	1	1

Hatching.—The percentage of eggs which hatch is remarkably high. From one group of six hundred eggs, five hundred and sixty nymphs hatched, or ninety-three per cent.

The hatching of the Odonate nymph is interesting in that it has a pronymphal instar between the embryo and the first active nymph.¹ In 1904 Pierre described the hatching from the egg, and the remarkable behavior of the pronymph of *Lestes viridis*. Balfour-Browne

¹The occurrence of a pronymphal instar in some species of Orthoptera is mentioned by Balfour-Browne (1909). And Smith (1920) records it for *Corydalis cornuta*.

followed in 1909 with a description of the phenomenon in *Agrion pulchellum* and *Ischnura elegans*. Tillyard (1917) gives an account of it for an anisopterous species, *Anax papuensis*.

In the present study, observations were made on the hatching of some thirty-five pronymphs of *I. verticalis*.² A pulsating organ was observed, corresponding to the so-called "cephalic heart." After reading Shafer's (1923) description of molting in *Anax*, I was impressed by the resemblance of these pulsations to swallowing movements. Since the pronymph is quite transparent, it was possible to see that the organ was in the proper position for the fore-intestine, and that the pulsations began anteriorly in the mouth region and progressed backward.

On one occasion, a pronymph was observed from the ventral surface (at magnifications of sixty and of one hundred and twenty) and the mouth was seen opening and closing rhythmically. The labrum, lying between the bases of the antennae, was being raised and lowered. From the frontal aspect, peristaltic contractions could be seen passing along the pharynx and oesophagus, corresponding in point of time to the movements at the mouth. The end view of another specimen, observed under high magnification, while the pronymph was still within the embryonic sheath, revealed muscular movements in the dorsal part of the head, coinciding with movements in the mouth region. These continued until the head filled the capsule. Shafer's (1923, pp. 321-324) assumption to the contrary, my impression of these dorsal contractions was that they were of muscles originating in the head exoskeleton, and inserted in the pharynx.³

The visible phenomena of hatching commence when the tip of the egg capsule appears above the plant stem. The operculum surmounts an expanding vesicle, as shown in Fig. 1, F.

The expanding vesicle is clearly not the whole egg, embryo and all, moving forward, but simply an anterior expansion of the shell filled with fluid. Tillyard suggested that the fluid might be blood—but if that were so, one should be able to see the corpuscles, for they are visible in transparent nymphs at a magnification of thirty. If it be only water, it must have been absorbed through the thin membrane of the vesicle by osmosis. Whether the vesicle represents merely the expanded egg shell, or whether the chorion has been ruptured, leaving the operculum stranded on top of an expanding

² And in addition, quite a number were killed in fixing fluid, for subsequent study.

³ Verified by a study of sections. See—Grieve, (1937).

embryonic sheath, is not satisfactorily established. The latter view seems more logical, and is partially supported by the fact that by careful manipulation of needles, the operculum could be made to slip around as if over the surface of the expanding vesicle.

Gradually the pronymph expands until the head fills the vesicle, as shown in Fig. 1, F. Here it is not a question of slipping forward bodily, but of stretching forward. Apparently the pronymph swallows the fluid which filled the vesicle, and uses it to produce the increased body pressure which causes the rupture of the embryonic sheath. In one individual the pulsations of the pharynx were counted. They numbered sixty-eight per minute and continued with decreasing rapidity for two minutes and thirty-five seconds. It is not certain whether the capsule always bursts in the same place, but sometimes, at least, the rupture is a dorsal longitudinal slit.

After the capsule bursts and the pronymph begins to slip out, swallowing movements again become more rapid. They continue until the pronymph is about half out of the sheath, or for about thirty seconds. Then the anterior opening of the digestive tube is closed (Fig. 1, J), and as the pronymph bulges out through the opening in the stem of the plant, the visible part of the digestive tube swells noticeably. A dorsoventral movement of the pronymph takes place, in a series of little jerks, accompanied by forward peristaltic contractions of the abdomen. This continues until the pronymph is about two-thirds out of the sheath, requiring about one minute and thirty seconds. Then, the sphincter at the posterior end of the mid-intestine seems to be closed. Rhythmic contractions arise in the hind-intestine, first in the vestibule, then in the rectum, and act with a pumping motion, forcing the distended mid-intestine forward like a piston. This is accompanied by forward peristaltic contractions of the abdomen, which tend to force the whole digestive tube forward, and which produce the pronounced thoracic hump (Fig. 1, L), a distinctive feature of the brief pronymphal period. At this time, the anterior end of the mid-intestine extends up into the metathorax, and the crop and gizzard fill the remainder of the thorax.

A dorsal longitudinal slit occurs in cuticula of the thorax, and the second instar nymph begins to swell out of the cleft. Gradually the sheath slips off the face; but the thorax is still humped and the head bent forward, until the labium is released.

The period from the splitting of the embryonic sheath until the splitting of the pronymphal sheath is considered as the duration of the pronymphal instar. It varied, in ten individuals that were

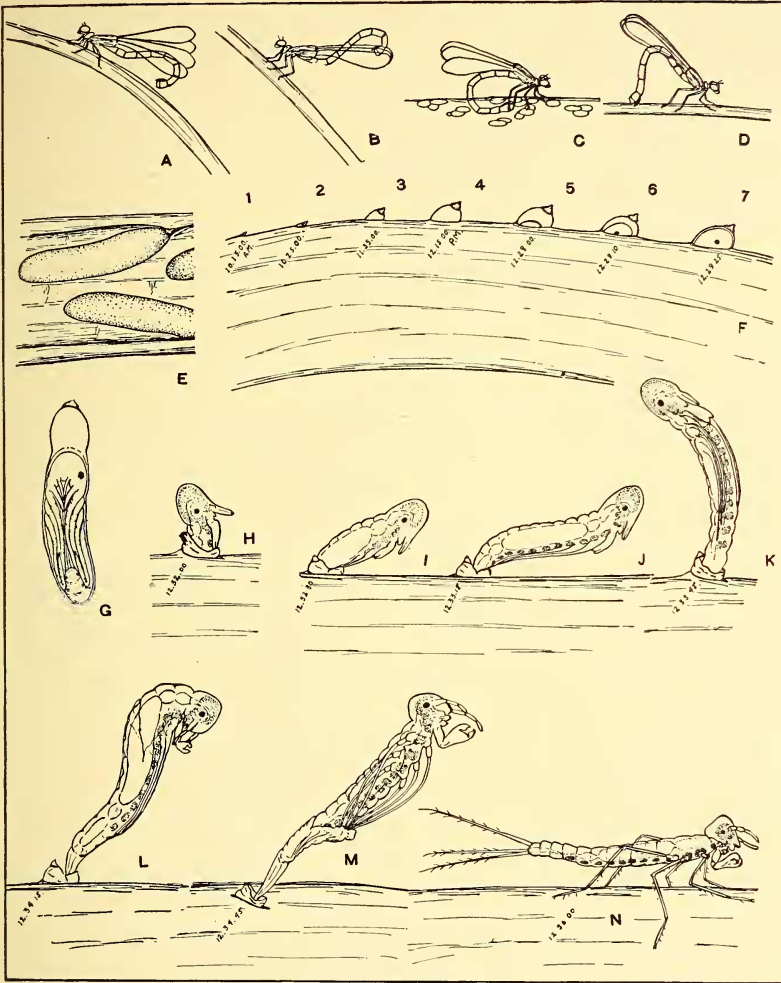


FIG. 1.—A and B, Positions assumed by mature unmated females, to attract males. C and D, Positions assumed by females depositing eggs. E, Plant stem opened to show eggs embedded in the tissue. F, 1 to 7, The egg capsule expanding and becoming visible above the plant stem, previous to hatching of the pronymph. G, Ventral view of the embryo at the stage represented in figures 4 and 5. F. H, I, J, K, Successive positions in the emergence of the pronymph. L, The "thoracic hump" phase, in which the pronymphal cuticula is ruptured, and the first molt commences. M, The second instar nymph emerging from the skin. N, The second instar, or first active nymph.

timed, from one minute fifteen seconds to two minutes fifty seconds, with an average of two minutes four seconds.

Following the bursting of the pronymphal sheath, the crop, gizzard, and mid-intestine still occupy the entire body cavity from the mesothorax to the end of the seventh abdominal segment. The rectum continues pumping while the mouthparts and legs are withdrawn from the sheath, with a little backward wiggling of the body. Then the nymph sways forward, places its feet on the plant stem, rests for a minute or less, draws out its caudal gills from the pronymphal sheath, and uses its feet to push off the cast skin.

The size and position of the various chambers of the digestive tract, relative to the body segments, varies slightly in different individuals. At about forty minutes after molting, the midintestine has come to occupy three and a half or four abdominal segments, extending from segment two to just past the middle of segment five. The yolk material then appears opaque, showing the outlines of the mid-intestine very distinctly, and constituting the "yolk plug" distinctive of very young nymphs.

Rectal movements are characteristic of young nymphs when at rest, and may have a respiratory function. They consist of three, four, or five rapid pulsations of the vestibule, and the water is then expelled by a forceful contraction of the rectum. The number of intake movements is subject to considerable variation. In six individuals timed, the counts ranged from thirty-four to forty-six per minute, with an average of thirty-nine.

The Pronymph.—The appearance of the pronymph before hatching is illustrated in figure 1, G (corresponding to figure 1, F, 4 or 5). The antennae lie close together, partially covering the mandibles and maxillae. The second maxillae are as yet not entirely fused to form the labium, and extend beyond the antennae. The three pairs of legs lie side by side, slightly curved, as shown. Those of the third pair, being longest, are folded back at about the junction of the tibia and tarsus, the latter lying forward below the tibia. The other two pairs lie straight, their termination marked by the slightly darker claws. The posterior abdominal segments curve ventrally, the tenth, ninth, and most of the eighth lying next to the shell on the ventral surface. The three long caudal appendages lie close together along the median line. They extend up under the labium, and are partially covered laterally by the first pair of legs.

There is a slight difference here from the condition in *I. elegans*, as described by Balfour-Browne. In that species, only the tenth

abdominal segment is turned forward, and the gills are said to lie between the legs and the shell. Another point of difference is that the pronymph does not emerge "in exactly the same position in which it has lain in the shell, the lamellae bent under the body, coming out points first." In *I. verticalis* it straightens out as it emerges, the tips of the caudal lamellae remaining within the egg shell even after the nymph has emerged from the pronymphal skin.

The pronymphal sheath surrounds each appendage separately, as was clearly shown by examination of cast skins floating on the water; and does not "closely follow the form of all the limbs, which are held down by it, close alongside the body," as described for *Anax papuensis* by Tillyard (1917).

The most conspicuous structure in the transparent pronymph is the opaque central nervous system. The brain and the three-lobed suboesophageal ganglion almost entirely fill the head. The bi-lobed ganglia of the ventral nerve cord extend along the ventral surface of the body like a row of buttons. There is a ganglion in each of the thoracic segments and in each of the first eight abdominal segments.

Since the pronymphal sheath appears to be of the same cuticular nature as all the other exuviae, there can be little doubt that the pronymph conforms with the accepted definition of a true larval instar. Hence the writer has followed Tillyard in considering the first active nymph as the second instar.

The Mechanism of Molting.—This subject has been very ably dealt with by Shafer (1923) for two representatives of the order, *Anax junius* and *Aeschna multicolor*; and by previous writers for other species of Odonata and Diptera. The present study of the molting and transformation of *I. verticalis* corroborates the main points of Shafer's hypothesis, but differs in some details. Such points of difference were checked on *Anax junius* and *Sympetrum vicinum*, and these were found to correspond with *I. verticalis*.

Some twenty-five or thirty specimens were studied. Most of them were killed in warm fixative, at various stages during and after transformation.

At the final transformation, the nymph always crawls out of the water, and remains so for some time before molting. During this time air must be swallowed, for specimens killed during the act of transformation had the *whole* digestive tract (not simply the mid-intestine) distended to almost fill the body cavity.

With reference to the morphology of the swallowing apparatus, previous authors seem to assume that the musculature of the fore-

intestine consists only of the circular and longitudinal muscles of the intestinal wall. But in addition to these there are, in *I. verticalis*, sets of dorsal, ventral and lateral dilators of the pharynx and buccal cavity, with their origin in the skeleton of the head.

The slit in the thoracic cuticula is preceded by abdominal contractions of a peristaltic nature, which probably tend to force the body fluid into the thorax, under the increased pressure produced by the distended digestive tract. Apparent swallowing movements continue meanwhile. Then, due to the "developmental preparatory weakening" along the middorsal line of the thorax, the cuticula ruptures in this spot, and the thorax of the imago begins to swell out of the cleft.

As the head is withdrawn from the nymphal skin, the cuticula of the fore-intestine is not drawn out through the mouth, as Shafer supposed, but becomes detached in the mouth region, and is subsequently swallowed. Hence the fore-intestine does not deflate at this time. Some individuals killed in fixative during transformation had the old cuticula loosened, but still lying against one wall of the still-distended fore-intestine. Just after the molt, the entire cuticula of the fore-intestine can be found, neatly folded in the anterior part of the newly formed peritrophic membrane. It is easily recognized by the chitinized teeth of the proventriculus.

Often the posterior end of this cuticula is still continuous with the anterior end of the peritrophic membrane of the previous instar. All of which is in accord with the recent findings (Wigglesworth, 1930, and Butt, 1934) regarding the structure and mode of formation of the peritrophic membrane.

In the hind-intestine, the cuticula is detached anteriorly (a sphincter closing off the mid-intestine), and as the molting insect slips forward out of the exuviae the cuticular lining is left behind in the cast skin. And yet, in certain of the dragonflies that were killed while the wings and body were still very soft, the rectum and pre-rectal ampulla were fully distended. Similarly in nymphs of *I. verticalis* that are very transparent just after molting, it can be seen that the hind-intestine is expanded in the same manner as the rest of the digestive tract. It might be refilled from the ventriculus, after the cuticula has been pulled out, or it might be filled by inhalation through the anus. The fact that in fixed specimens the pre-rectal ampulla was sometimes contracted although the rectum was distended, tends to indicate that the latter was refilled through the anus. The hind-intestine is then either held in a distended condition during transformation and while the body and wings harden, or it

may have a pumping action during actual transformation, similar to that of the pronymph. It may also be distended previous to the molt, as in the case of the pronymph. The presence of dilator muscles of the vestibule,⁴ acting in combination with the sphincters, makes it possible to draw in water at this end of the digestive tube.

Hence I would revise Shafer's hypothesis of the mechanism of molting as follows:

The nymph swallows water, or air, until the *whole* digestive tube becomes distended. The blood pressure is consequently increased. Abdominal contractions of a peristaltic nature proceed from the posterior end forward, decreasing the lumen of the abdomen, and concentrating the body fluid, under pressure, in the thorax. The rupture of the thoracic cuticula takes place subsequent to these contractions of the abdomen (which often are accompanied by apparent swallowing movements). As the thorax swells out through the cleft in the cuticula, the pressure in the head and legs is reduced, and they can be drawn out. When the head and thorax have reached their full size, the insect places its feet on the support and draws the abdomen out of the exuviae. A considerable additional quantity of air must be swallowed after the insect has come out of the exuviae, in order to stretch the body and wings, and maintain the tension until the chitin has hardened.

Thus by swallowing water, or air, to increase the volume of the *whole* intestine, the muscular pharynx furnishes an important part of the motive power for emergence. Additional power is furnished by the abdominal contractions.

Rearing Procedure.—The most successful containers used for rearing young nymphs were the small "boats" pictured in figures A and B, of Plate III. It was necessary, if the nymphs were to retain all their appendages, that they be kept in separate vessels. The frame of the boat was of balsa wood which is very light and buoyant, and the "hold" of the vessel was made of silk, the meshes of which were small enough (144 threads to the inch) to confine both the nymph and its prey, while permitting the advantages of the more even temperature of a large vessel of water (figure B, of Plate III). The silk was fastened to the frame with paraffin, and the frame covered with a film of paraffin to prevent "water-logging." The little "sail" was a tag used to keep a record of the dates of molting.

During the eighth, ninth, or tenth instars, the nymphs were transferred to tall glasses (figure C, of Plate III), provided with a piece

⁴ Described by Whedon (1918) for other members of the Order.

of wire screen to serve as a perch. Previous to transformation the glasses were covered with cheese-cloth, fastened down with an elastic band, thus making convenient emergence chambers in which to observe the changes taking place. Aquarium cages (figures E and D, of Plate III) were used for individual adult specimens whose records were to be kept, and similar, larger cages for stock adults. The cage was made of wire screen, fitted to the dimensions of the aquarium. The opening for admitting specimens, food, etc., was protected by a cheese-cloth sleeve.

After an unsuccessful trial of *Paramecia* as food for the very young nymphs, Chironomid larvae were used, and proved satisfactory for nymphs between the second and seventh instars. The method was to collect Chironomid egg-masses and, when they hatched, to transfer the newly hatched larvae with a pipette to the "boats" in which the nymphs lived.

From the sixth or seventh to the ninth or tenth instars, the nymphs were fed *Ceriodaphnia*, and *Daphnia pulex*; after the ninth or tenth, the larger *Daphnia magna*.

The adults, as they emerged, had to be fed living insects, preferably Diptera. One method was to stock the tank of the rearing cage with large blood worms, so that the damselflies could feed on the emerging midges. Mosquito larvae and pupae were also introduced. But it often became necessary to supplement these with midges collected with a net in the woods.

The Nymphs.—The grown nymph of *I. verticalis* has been described by Needham (1903), Garman (1917, 1927), and Howe (1921). The accompanying figure (Plate I, A) of a nymph in the early eleventh instar, illustrates the external structure, and such pattern as is common to all nymphs.

In most respects the younger nymphs resemble the mature ones, but each instar has fewer specializations than the succeeding one. Previous developmental studies on various Odonata have shown that characters of labium, antennae, tarsi, genitalia, and wing buds, furnish means of differentiating successive instars. The accompanying table summarizes such characters. Inspection of it will show that instars II to VI are readily distinguishable, but that because of the variability of the mental setae, it is impossible to separate with certainty instars VII, VIII, and IX. Instar X can be distinguished by the number of the lateral setae. In the cases of XI and XII, supplementary measurements of various dimensions are useful, but not infallible.

TABLE I.—DIAGNOSTIC CHARACTERS OF NYMPHAL INSTARS
Instars II to XII

Character	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Ant. seg.	3	4	5	5	6	6	6	6	6	6, 7	7
Trs. seg.	1	1	1	2	2	3	3	3	3	3	3
Lat. set.	1	2	2	3	3	4	4	4	5	6	6
Ment. set.	0	0	1	2	$\left\{ \begin{array}{l} 2, \\ 2+1 \end{array} \right.$	$\left\{ \begin{array}{l} 2+1, \\ 3 \end{array} \right.$	$\left\{ \begin{array}{l} 3, \\ 3+1 \end{array} \right.$	$\left\{ \begin{array}{l} 3, \\ 3+1, \\ 4 \end{array} \right.$	$\left\{ \begin{array}{l} 3+1, \\ 4 \end{array} \right.$	$\left\{ \begin{array}{l} 4+1, \\ 5, \\ 5+1 \end{array} \right.$	$\left\{ \begin{array}{l} 5, \\ 5+1 \end{array} \right.$
Wing buds	0	0	0	0	vis.	rud.	rud.	inc.	inc.	inc.	inc.
Body l.* (ave.)	1.18*	1.6	1.9	2.6	3.8	4.6	5.2	6.6	9.0	11.9	12.5
% inc.		35.6	18.8	36.8	46.2	21.1	13.0	26.9	36.4	32.2	5.1
No. spec.	20	18	13	14	11	8	12	12	10	22	8
Body l.* (max.)	1.30	1.90	2.17	2.79	4.28	5.10	5.71	7.95	10.54	14.30	15.0
% inc.		46.2	14.2	28.6	53.4	19.2	12.0	39.2	32.6	35.4	4.9
Body l.* (min.)	.99	1.30	1.77	2.23	3.16	3.94	4.76	5.78	7.34	10.0	10.9
% inc.		31.3	36.2	26.0	41.7	24.7	20.8	21.4	27.0	36.2	9.0
Gill l.* (ave.)	.96	1.2	1.6	1.9	2.5	3.2	3.7	4.2	4.7	5.9	6.0
% inc.		25.0	33.3	18.7	31.6	28.0	15.6	13.5	11.9	25.5	1.7
Head wid.* (ave.)	.30	.43	.54	.75	1.0	1.3	1.55	1.85	2.1	3.1	3.3
% inc.		43.3	25.6	38.9	33.3	30.0	19.2	19.4	13.5	47.6	6.5

Abbreviations:

Ant. seg. Antennal segments.

Trs. seg. Tarsal segments.

Lat. set. Lateral setae of labium.

Ment. set. Mental setae of labium; 2 + 1, *etc.*, = 2 long, 1 short.

vis. Barely visible.

rud. Rudimentary.

inc. Increasing in size.

No. spec. Number of specimens on which averages are based.

* Measurements of length and width are in millimeters.

Growth.—Measurements on body length were made daily on five nymphs during instars II to VII, to determine the increase between molts as well as at the molt. It was found that there was a definite

progression in size as the instar advances, followed by a slight step up at molt.

The data on size, recorded in Table I are the maximum, minimum, and averages of measurements of a collection of nymphs chosen indiscriminately as to age, except that there were always some recently emerged, some on the verge of molting again, and some intermediate ones. In order to get an estimate of the rate of growth from instar to instar, the percentage of increase was calculated from the measurements given in the table. In every case, the percentage of increase fluctuates, as though growth occurred in cycles, rather than at a constant rate.⁵ But there was no very marked agreement between the percentage of increase of the various structures measured, in any instar.

Hence, in order to determine whether sets of measurements made at the same period in each instar would give more constant rates of growth, exuviae were examined—ten for each instar. Measurements were made on width of mentum and length of hind femur. These are sufficiently rigid structures to eliminate error from wrinkling or collapse. The results, given in Table II, show a similar inconstancy.

TABLE II.—MEASUREMENTS OF PARTS OF EXUVIAE
Instars II to XI

	II	III	IV	V	VI	VII	VIII	IX	X	XI
Width of mentum*	.48	.64	.82	.95	1.18	1.49	1.96	2.36	2.79	2.99
% increase		33.3	28.1	15.9	24.2	26.3	31.5	20.4	18.2	7.2
Length of femur*	.68	.95	1.23	1.54	1.84	2.35	3.05	3.77	4.47	4.99
% increase		39.7	29.5	25.2	19.5	27.7	29.8	23.6	18.6	11.6

* Measurements in millimeters.

Factors Affecting Length of Instars.—The duration of any instar is subject to the influence of a number of factors, the most obvious of which are temperature, food, and disease. My object was to simulate normal optimum conditions—as nearly as possible. It was comparatively easy to supply the nymphs with all the food they would eat. To avoid excessively high temperatures, the aquaria

⁵ Balfour-Browne (1909) distinguishes between “growth moults” and “developmental moults.”

were kept in a cool part of the insectary in hot weather, and they were moved out under the glass in the greenhouse when the weather was cool. The influence of disease was probably the most important factor affecting the period between ecdyses.

During the latter part of August there was a noticeable lengthening of the instars. Early in September the morality increased alarmingly. On September 20 inspection of a number of the dead nymphs revealed that in almost every one the post-abdomen was darkened. Upon dissection, each of these had three black plates in the rectum, corresponding to the pads of specialized tissue that are sometimes called rectal "glands." Evidently this tissue is subject to some disease which may be fatal to the nymph.

The diseased condition could be noted in the living nymphs as well, and a record was kept of their behavior. Sometimes after a molt the nymph seemed to be normal again and recovered. Otherwise death usually occurred within a day or two after the diseased nymph had molted.

Duration of Instars.—The object of rearing a relatively large number of individuals was to determine the average length of each instar, and to insure having some of them complete the life cycle. Table III shows the data obtained. The instars are arranged horizontally. The ordinates are the instar periods, and the plotted figures represent the numbers of individuals which molted within a given number of days. There is a fluctuation in totals in the early instars, due to the occasional loss of the cast skins, whereby the data on the instar period were lost, for the preceding and succeeding instars.

It will be seen from inspection of these data that the first five instars show normal curves, sloping up sharply to a single peak, and down again a little more gradually. These instars presumably represent an almost normal condition, with the most nearly optimum in the fifth, whose range is narrowest. The slightly longer period of the second instar, since it is uniform, must be influenced by some one factor, affecting all the individuals.

A possible unfavorable factor might be the inaccessibility of food; for even newly hatched Chironomid larvae were almost as large as the infant nymphs and therefore difficult for them to manage. However, in the last lot in the experiment (a group of nineteen) the second instar period was shortened to a mean of 6.3 days, probably because of improved technique and feeding facilities. From which one may infer that if optimum conditions could be secured, the second instar would not be longer than the others.

TABLE III.—DURATION OF NYMPHAL INSTARS

Vertical columns are the number of individuals per instar in each day-group
Lower table gives the mean length of instars

Period in days	INSTARS									
	II	III	IV	V	VI	VII	VIII	IX	X	XI
2		2	1		1					
3		16	7	15	5	4	1			
4		26	45	33	19	6	2	4	2	
5	4	21	29	28	24	21	8	8		
6	14	8	6	8	18	8	9	6	1	
7	23	5	2	3	11	10	9	4	6	
8	20	3			3	7	6	2	4	1
9	14	4	1			4	4	2	3	1
10	10				1	2	8	4	2	1
11	5					4	5	5	4	1
12						6	3	3	2	3
13						1	3	2	1	6
14							1	2	2	
15						2		3	2	
16							1	1	1	
17	1						4	2	1	1
18							2		1	1
19							1			
20							2			
21								1	2	1
22								1	1	
23							1	1		
24								2		
25								2		
26									1	1
27										
28										
29						1				
30										
31										
32										
33										1
Total no. individuals	91	85	91	87	82	76	70	55	36	81
Mean in days	7.8	4.8	4.5	4.4	5.3	7.1	7.9	10.9	11.5	13.7
σ	1.7	1.6	1.1	.97	1.4	2.9	4.8	6.0	5.2	4.3
S. E.	.18	.18	.11	.10	.16	.34	.59	.81	.87	1.0

The later instars, seven to eleven, show irregular curves, and prolonged periods, indicating the influence of one or more unfavorable factors. And the irregularity coincides with the outbreak of disease.

The very low error in proportion to the mean in instars II to VI shows the significance of these figures. In the later instars the de-

creasing number of individuals makes the value of statistical treatment doubtful, but the data are included to complete the present record.

However, to show that the average period is fairly uniform for all stages up to the penultimate, I present the record of eight individuals which I think may be considered normal. These were a group that maintained steady growth throughout, transforming early in September, at the end of eleven instars. Apparently they escaped the disease entirely. The data for these nymphs are given in table IV. There is nonconformity in the second instar as in all other individuals of the same age. But the following instars show a nice degree of uniformity, with a slightly lengthening period in the later instars, and a noticeable increase in X. The ultimate instar is remarkably constant at twelve to thirteen days. In these eight individuals, the total nymphal period ranged between fifty-seven and sixty-four days.

TABLE IV.—DURATION OF NYMPHAL INSTARS OF EIGHT NORMAL INDIVIDUALS
Vertical columns are number of individuals per instar in each day-group

Period in days	INSTARS									
	II	III	IV	V	VI	VII	VIII	IX	X	XI
3		1	1	2	1	1				
4		3	4	3	3	4	2	1	1	
5		4	3	3	3		2	5		
6	1				1	2	4	1		
7								1	2	
8	2					1			3	
9	4								1	
10	1									
11									1	
12										3
13										5
Average in days	8.5	4.4	4.3	4.1	4.5	5.0	5.3	5.3	7.8	12.6

Variation in the Number of Instars.—There were eight other nymphs, with a record of disease in August and September, in which the whole nymphal period was extended beyond the normal time for transformation. These all molted into the twelfth instar. One died from disease. Six others, finding themselves in an environment that continued the warm temperatures and abundance of food of summertime on into November and December, finally transformed

after a much prolonged ultimate instar, viz., thirty-eight to sixty days. Hence it would appear that eleven is the normal number of instars for the summer generation, and that twelve⁶ or thirteen is common for those (nearly mature ones) that live over the winter. One individual, however, molted into the thirteenth instar in October, after nineteen days in the twelfth, and died shortly after from disease.

Mortality.—The mortality figures are based on the actual number of nymphs in the experiment, beginning with 120. A few of the dead or missing were replaced at first, if individuals of the same age were still available.

The mortality due to so-called natural causes and accidents decreased from fifteen percent in the second, to three percent in the fifth. This would be expected as the nymphs grow stronger and better able to cope with circumstances. Obviously these nymphs were protected from adverse conditions to a much greater extent than in their natural habitat.

The mortality rate in instars VI and VII was about stationary at six percent, but VIII began to show the effects of disease. Thereafter the rate increased to twenty percent, thirty-five percent, and fifty percent when the epidemic reached its peak.

INSTARS II TO XI

	II	III	IV	V	VI	VII	VIII	IX	X	XI
Total	120-	110-	98-	92-	89-	83-	78-	71-	54-	36-
nymphs	102	98	93	89	83	78	71	55	36	18
% mort.	15	11	5	3.2	6.7	6	9	20	35	50

*Pre-transformation Changes in the Ultimate Instar.*⁷—Early in the ultimate instar, the eye is similar in shape and position to that of the preceding instars (Pl. I, fig. A). Careful examination under the binocular shows that a considerably greater area of cuticula is marked off in minute facets (fig. B). Bands of black pigment (present also during several previous instars), are visible, as shown, extending to the margin of the faceted area. The ventral aspect is quite similar to the dorsal. The ocelli are inconspicuous at this time. Their position is marked by transparent areas in the cuticula.

⁶ Overwintered nymphs molt at least once in the spring, before transformation.

⁷ This record taken from those nymphs that transformed at the end of eleven instars.

Usually about the fifth day, the conspicuous black part of the eye becomes somewhat longer, by increase along the inner margin, making the whole eye appear less lateral, and more frontal (Pl. I, fig. C). At the same time there are slightly darkened crescents in the region of the ocelli, and sometimes it is possible to see their nerve trunks leading from the brain.

By the sixth or seventh day the eyes have assumed their oblong shape and frontal position. There are also certain changes in the shape of the whole head (Pl. I, fig. D). The altered external appearance of the eye is due no doubt to the growth and pigmentation of a great many new ommatidia, that will constitute a considerable part of the functional imaginal eye (Lew, G. T., 1934). By this time the ocelli are distinct, brownish, crescent shaped structures, beneath the cuticula (fig. D).

Immediately following the development of the oblong eye, the wings change from their blade-like shape and overlapping position to a more cylindrical shape and a parallel position.

Usually during the two or three days before transformation a certain definite color sequence can be noted. The wings take on a pale orange or brownish tint, and the thorax becomes brownish. The colors gradually deepen, and the day before transformation, the whole nymph is an orange-brown, the thorax darkest, sometimes forecasting the imaginal pattern; and the thoracic spiracles are conspicuously black.

Several hours previous to the last molt the nymph climbs partially out of the water, either vertically with the head and thorax out, or horizontally, with one side of the thorax out. Sometimes this behavior was noted in the afternoon, in which case the nymph would transform the following morning. This probability indicates that the caudal gills have become non-functional, and that direct respiration has commenced, making first use of the thoracic spiracles.

THE ADULT

Réaumur (1734-42) gave us the first recorded description of the emergence of the odonate imago. Since then the phenomenon has been described for various members of both Anisoptera and Zygoptera. The thesis (in Cornell University Library) of which this paper is a condensed version, contains a detailed description of the transformation of *Ischnura verticalis*.

Female Polychromatism.—There are records in the literature of at least four color variations in the adult females of *I. verticalis*, with the male resembling one of them. The predominant colors of

the male are green and black, with a conspicuous blue marking on the tip of the abdomen. The four color forms of the female may be summarized as follows:

- Orange Forms: (1) "Heterochromatic" of Calvert,^s with blue-tipped abdomen.
 (2) "Orange variety" of Garman^s and of Lyon,^s with dorsum of segments four to ten entirely bronze.
- Dark Forms: (1) "Black variety" of Williamson^s and of Garman, like the male, but with dorsum of abdomen entirely dark.
 (2) "Homeochromatic" of Calvert; "blue-tipped" variety of Garman; "green form" of Ris;^s resembling the male.

Note: For additional descriptions of adult females, see: Say (1839); Hagen (1861); Selys-Longchamps (1876); Calvert (1893); Walker (1908); Garman (1917), and Needham and Heywood (1929). All agree that these forms all become pruinose with age.

Of these four types of females, *only* the second, the non-blue-tipped orange variety seems (with a single individual exception) to be present at Ithaca, New York. This fact leads to the suggestion that the color differences may represent different geographic races, for it will be noted in the literature that the specimens described are from various parts of North America, over an extensive geographic range.

In the vicinity of Ithaca, N. Y., specimens showing varying degrees of confluence of the postocular spots are not uncommon and there is one specimen in the Cornell collection (collected at Ithaca in 1897), in which the postocular spots are entirely isolated from the pale color of the occiput. In this specimen the pale color is greenish brown and the dorsum of the abdomen is dark on all segments. It is, no doubt, the "black variety" of Garman and of Williamson, and it appears to be the only representative of any type other than the "orange variety" of Garman and of Lyon recorded from this vicinity. In the same individual, the antehumeral pale stripe is interrupted, forming an inverted exclamation mark, as in *I. posita*. This latter variation was also occasionally noted among the specimens reared for experimental work. It occurred in both males and females.

^s Calvert (1915); Garman (1927); Lyon (1915); Williamson (1900); Ris (1903).

For observation on developmental changes in female coloration, adults were kept in aquarium cages (Pl. III, figs. E and D), both collectively and singly. They were fed daily, allowed to mate and oviposit, and were under constant observation during their entire adult life. Their color changes were noted carefully, especially during and immediately following transformation, and in no case was the abdomen of the female blue-tipped, nor were any specimens colored like the male.

During and after transformation, the color sequence is as follows: At first pale all over, until the imago has attained its full size (requiring three-fourths of an hour after actual emergence). Gradually the dark parts of the thorax assume an olivaceous hue, while the light parts are still buff. Then the dorsum of the abdomen becomes slightly olivaceous, an orange tint can be seen on segments one, two, and three, and on the thorax. After about one hour the imago begins to walk about and soon after is able to fly. A half hour later, the thorax is ochre yellow and dark brown, the dorsum of abdomen ochre yellow on segments one, two, and part of three, and dark olivaceous on the remainder. In some specimens there are light buff areas on the posterior margins of eight and nine, or only of eight. After two hours the colors are all somewhat darker. After four more hours, or about seven and one-half hours from emergence, the colors are mature, *i.e.*, orange and bronze on the thorax, bronze on the dorsum of segments four to ten, with light strips on eight and nine (when present) scarcely noticeable.

The blue tail pattern of the male is developed gradually, appearing orange at one hour from emergence, grey after four hours, pale blue after six and one-half hours, and mature blue the following day.

It is not true that blue is present early in the life of orange females of this type, and later disappears. It must certainly be a condition present in some, and absent in others. It might even be, since in the ontogeny of the male orange or buff is a forerunner of blue, that those females with buff or tawny bands on eight or nine are intermediate forms in the evolution of the blue-tipped females.

After wearing these colors for four to seven days, the orange becomes distinctly darker, and more reddish, followed by very dark brown, and at the same time the metallic bronze-black becomes dull, lustreless black. This transition phase has a duration of from one to two and a half days. Then the individual becomes pruinose, *i.e.*, the orange markings are replaced by bluish-grey and the bronze areas become pollinose. The pruinose phase lasts for the remainder of adult life, which may be several weeks.

Dissection of females of various ages showed the ovaries immature in the earliest imaginal state, while, at the time of color change, one egg at the end of each ovarian tube or egg string is mature. This fact explains the various records in the literature of slender abdomens in the orange variety, and plump abdomens in the pruinose ones. Oviposition never occurred before the color change, but usually directly after, hence color change is evidently more or less coincident with sexual maturity.

The fact that imagos may live for over a month, and are in the pruinose phase for three-quarters of that time, accounts for the predominance of these individuals in the latter part of the season.

No doubt the generations overlap considerably, with more or less continual emergence of adults from May to August, but the cycle is probably as follows: Adults emerge in the latter part of May from overwintered nymphs, and adults emerge in early August from summer nymphs to produce the next crop of winter nymphs.

Mating and Oviposition.—Mating usually takes place during or just after the color change of the female, namely between the fourth and eighth days.

The courtship behavior of unmated, mature, females is more conspicuous than that of the males. The female, perched on a blade of grass, with the post-abdomen flexed ventrally (as shown in fig. 1, A and B) and wings fluttering, will, on the approach of a male, commence a vigorous fanning with her wings, to attract his attention.

Oviposition may begin within a few hours after mating, or not for several days thereafter. I have records of first oviposition varying up to ten days after transformation. Some females have been known to deposit eggs unfertilized. Plate I, figs. C and D show the positions assumed by females while depositing eggs.

As many as four hundred eggs may mature simultaneously (*viz.*, the terminal ones of all egg-strings of both ovaries), and are deposited in the course of a few hours. Then after an interval of about five days another lot is matured and deposited; and this procedure may be repeated several times. I have a record of one veteran female which lived thirty-four days of adult life, and deposited eggs on five successive occasions, although the last lot, which was laid just before she died, was incomplete. Her total egg production amounted to approximately sixteen hundred fifty eggs. This female was fertilized but once, yet all the batches of eggs hatched.

PARASITES

Trematodes.—While nymphs were being dissected, small flatworms were frequently noticed in the haemocoel. The fluke popu-

lation then became a matter of interest, and a preliminary investigation was undertaken. Both nymphs and imagos of *I. verticalis* are subject to invasion by certain immature stages of two quite different trematodes, which may be described as follows:

Fluke no. 1 is a tailless Distome cercaria, occurring in the haemocoel of nymphs and adults. Living specimens measure approximately 1.5 mm. long, by 0.15 mm. wide, when fully extended, and about 0.55 mm. \times 0.19 mm., when contracted. The Y-shaped excretory canal is conspicuous. Oral and ventral suckers are present; the former measuring about 0.07 mm. and the latter 0.05 mm. in diameter. The piercing organ, if present, was not observed.

A similar fluke, designated as Fluke no. 1A, a metacercaria of a Distome, was found encysted in the respiratory tissue of *Anax junius* and *Sympetrum vicinum*. When the metacercaria escapes from the cyst, it moves about actively, by means of its two suckers. The living individual is indistinguishable from Fluke no. 1. The size is approximately the same. The following description is from preserved and stained material. There is an anterior oral sucker measuring 0.07 mm. to 0.08 mm. in diameter. The oral cavity opens into a short muscular pharynx about 0.05 mm. wide (Pl. II, G.) The esophagus is extremely short, dividing at once into a pair of intestinal coeca, which extend back to within an eighth of the body length from the posterior end. The Y-shaped excretory canal is dorsal to the intestinal coeca where they cross, but it seems to be somewhat ventral at the extremities. The ventral sucker is situated slightly caudad to the middle of the body, just posterior to the forking of the excretory canal, and measures 0.05 mm. in diameter.

Rudimentary gonads are present. The paired testes are located ventrally, behind the ventral sucker, and between the coeca, just posterior to the forking of the excretory canal. The left testis is slightly anterior to the right one. The ovary lies slightly to the left of the ventral sucker. Its extent is variable, and it is rather indistinct.

Attempts at vital staining with neutral red have thus far been unsuccessful in demonstrating the penetration glands, but in some of the living material and in some preserved specimens it is possible to distinguish a structure which is indicated in Pl. II, G, just in the crotch of the intestinal coeca. Whether this represents the remains of the penetration glands, or whether it is a rudimentary uterus, could not be ascertained. It seems to have a duct leading to the exterior at the anterior end of the animal, to the right of the oral sucker.

The above description, although incomplete, seems sufficiently similar to descriptions of other metacercariae recorded from Odonate hosts, to warrant placing this form with them, in the family Plagiorchiidae.

Fluke no. 2 is also a tailless Distome cercaria. It occurs within the mid-intestine of both nymphs and adults of *I. verticalis*. Living specimens exhibit two conspicuous characteristics. There is an enormous bowl-shaped ventral sucker (see lateral view, in Pl. II, J2). And the excretory canal appears to be an inverted V-shaped structure, which probably represents the union anteriorly of the cornua of a Y-shaped canal, whose posterior portion is inconspicuous. The supposition is substantiated by a fairly definite median opening at the caudal extremity, in some of the preserved specimens. This type of excretory canal is said to be characteristic of only one family of Digena, the Hemiuridae.

The extended, living cercaria measures about 1 mm. in length, with proportions as indicated (drawn to scale) in Plate II, K. The oral sucker, which is sub-apical, measures about 0.14 mm. \times 0.07 mm.; the large ventral sucker is 0.22 mm. in diameter, situated just behind the middle of the body.

The oral cavity opens into a muscular pharynx, which in turn leads into a sac-like oesophagus. The intestinal coeca do not open directly out of the oesophagus, but are connected with it by means of a pair of lateroventral, bulb-like structures, which leave the oesophagus about midway of its length (see Plate II, L). The intestinal coeca are narrowed in the region of the ventral sucker, and they extend almost to the posterior extremity of the body.

The gonads appear as very rudimentary structures. The paired testes are ventrally located behind the ventral sucker, toward the lateral margins of the intestinal coeca, the right one slightly anterior to the left. A median structure near the posterior end of the body probably represents the ovary.

Occurrence and Infestation—From July 10 to August 10 cercariae of Fluke no. 1 were found in the haemocoel of nymphs and adults of *I. verticalis*, although with decreasing frequency. Nymphs commonly harbored six to eight flukes, but there were seldom more than two or three in imagos. This fact may be an indication of a lethal dose. After the middle of August the flukes disappeared.

Meanwhile the encysted metacercariae, mentioned above as Fluke no. 1A had been discovered in great abundance in nymphs and imagos of the large Anisopteran dragonflies, *Sympetrum vicinum*

and *Anax junius*, and to a limited extent in *Libellula pulchella* and *Plathemis lydia*—all collected from the same pond from which the infected *I. verticalis* were taken.

In these larger nymphs, the cysts were always found within the tracheal gills of the branchial chamber. There seems to be a proliferation of host tissue, in the nature of a gall, surrounding the trematode cyst. When carried over into the imaginal dragonflies, the cysts undergo a change of location. The branchial basket is no longer used for respiration when the insect becomes aerial, and the rectum is consequently greatly reduced. So, in the adult dragonflies, the cysts are found thickly clustered around the reduced rectal region, held together by a connective-tissue-like structure.

This trematode seems always to select a location within the host that will ensure it a ready supply of oxygen. In the older imagos it was especially noticeable that each cyst was connected with at least one fairly large trachea, and sometimes the cysts were located somewhat away from the rectal region. Plate II (figure H) illustrates this remarkable response by the host to the trematode invasion. Tracheal ramifications completely envelope the cyst in a glistening white sheath, or gall. The figure shows two galls in the process of formation, one small gall from which the cyst has been removed, and one large gall showing the extensive ramifications of the tracheae. Occasional bulging of the sides of the gall indicated that the fluke was moving inside, and when this gall was opened, it revealed a thin-walled cyst of the same proportions as the others in the figure, namely about 0.3 mm. in diameter.

It is important to state that in all cases of encystment, when the galls were opened carefully with dissecting needles, the thus liberated metacercariae were enclosed in a thin transparent sheath, or cyst wall. None were found with the thick striated wall possessed by a percentage of the specimens recorded by Szidat (1926), Ono (1928), and Kotlan and Chandler (1927), for various *Prosthogonimus* sp. taken from odonate hosts.

The significance of this point did not appear until the paper by Ono came to hand, which was only after some attempted infection experiments on domestic chickens had produced negative results. Ono observed that only the metacercariae from thick walled cyst produced infection. It may be that the thin-walled transparent cysts developed into the ones with the thick striated walls, or that the above mentioned authors have been dealing with more than one kind of trematode, as suggested by Kotlan and Chandler (1927).

Regarding the possible rôle of the damselflies in the life history of the fluke, assuming that Fluke no. 1 and Fluke no. 1A might be different stages of the same species, it has been noted above that long after the cercariae ceased to be present in the damselflies, the similar, encysted metacercariae continued to be abundant in dragonflies. In fact, infected nymphs were taken from the pond as late as December. It is possible that dragonflies could be infected by eating infected damselflies. This hypothesis is especially plausible since the larger dragonfly nymphs prefer insect prey to the small plankton organisms which form the diet of damselflies.

Szinitzin (1907) records certain cercariae, having a form and mode of locomotion resembling *Culex*, being eaten by *Agrion* and *Epitheca* and producing infection in the latter. Hence it is theoretically possible that *Ischnura*, and perhaps *Anax* also, could be infected in this manner.

On the other hand Krull (1931) reports "active" infection by the cercariae of *Pneumonoeces medioplexus* and *Pneumobites parviplexus*. These cercariae enter the rectum, caught by the respiratory currents, and there they penetrate the gill tissue.

The occurrence of Fluke no. 2 was first noted on July 16. It continued to be present until August 24, although with decreasing frequency after August 17. Of the sixty-two nymphs and adults of *I. verticalis* in which the presence or absence was noted, fifty-five percent were infected. The infection rarely exceeded six parasites per host.

A Green Flagellate—The nymph infected by this protozoan may often be noted waving the tip of the abdomen back and forth in a restless manner. If the nymphal cuticula is transparent, microscopic examination shows that the interior of the posterior end of the abdomen is dark greenish. The instar period of infected nymphs is always longer than that of normal individuals. When the infected nymph molts or transforms, the rectal cuticula of the cast skin contains a multitude of unicellular green organisms.

Within two days the nymph again exhibits the uneasy, waving movements of the abdomen. Closer examination shows three elongate blackish structures within the posterior abdomen. These are the diseased "rectal pads." Sectioned material shows that the rectal pads are badly disintegrated after infection, in spite of the fact that the protozoans do not penetrate the cuticula, but remain in the lumen of the rectum.

In the majority of cases the nymphs die before the fourth day after the molt. Occasionally they recover, if the infection has been slight.

Studies were made of the living material in many stages of the life cycle as possible. Scarcely any two nymphs harbored protozoans of exactly the same phase of development. Both sessile and motile forms occur. The former may be attached to the cuticular lining of the rectum of the nymph, or may be free in the lumen of the rectum. The shape may be globular or ovoid; the individuals may be separate, or in clusters loosely attached to a central matrix. The ovoid individuals exhibit limited metabolic movement. The periplast is transparent and colorless, and there is an irregular peripheral structure, diffusely green, with clear granules distributed through it. The nucleus is central and colorless in living material, but deeply staining in sections. A pair of reddish-brown pigmented bodies is present, centrally or eccentrically located (Plate II, C). Asexual reproduction in the sessile stage is by fission or multiple cell formation, (Plate II, B1 and B2).

The motile forms were obtained when ovoid organisms from cast skins were kept for one-half hour to several hours on a slide in a moist chamber. On several occasions individuals changed from the nonmotile to the motile phase while under observation. The motile form is a flagellate. Only one flagellum was seen, and it was longer than the body. The form is elongately cylindrical, with transparent periplast prolonged into an acute process posteriorly. The cell contents are green peripherally, as in the sessile forms, with clear granules distributed throughout. A transparent central nucleus, and a pair of dark pigmented bodies are present. Anteriorly a clear area seems to represent a gullet, opening into an anterior reservoir.

Other Parasites or Epizoids—Unidentified gregarines were frequently found in the lumina of the mid-intestines of nymphs and adults of *I. verticalis*. They lie between the peritrophic membrane and the intestinal wall, with the anterior tip of the organism buried in the epithelium. About forty percent of the insects examined for parasites were infected with gregarines, varying from one to twenty or thirty per damselfly. In about six percent of the nymphs examined, round, opaque, white cysts were found.

During mid-season it was common to find nymphal stages of aquatic mites attached to the *Ischnura* nymphs. Their favorite places of attachment were the ventral conjunctiva of the thorax, the bases of the legs, or occasionally the region between the wing pads

and the body. About thirty percent of the nymphs examined were infected, as many as twenty mites sometimes being present on one host. Through the kindness of Dr. Ruth Marshall, these mites have been identified as belonging to the genus *Arrhenurus*.

A discussion of the parasites of *I. verticalis* would not be complete without mention of *Hydrophylax aquivolans* a minute Trichogrammid egg-parasite, described by Matheson and Crosby (1912).

SUMMARY

In this paper life history data have been compiled on all stages of the development of *I. verticalis*, beginning with one hundred twenty eggs, and ending with thirteen adults.

The phenomenon of hatching, repeatedly observed, was found to be analogous with molting and transformation. The myth of a transient "cephalic heart" in the pronymph is replaced by the explanation that water is swallowed into the digestive tract, thus increasing the body pressure and assisting in the rupture of the embryonic or nymphal sheath.

Diagnostic characters of the instars are described. Measurements were made on various body structures from instar to instar, to study growth. The duration of nymphal instar has a mean period of about five days under optimum experimental conditions. In the later instars, many of the nymphs were affected by a disease which produced a pronounced lengthening of the instars period and an increased mortality.

The subject of female polychromatism is discussed, and the fact established that only one of the four known color varieties is present in the Ithaca fauna.

A brief study of the parasites of *I. verticalis* reveals a green protozoan, frequently fatal to the nymph inhabiting the rectal cavity; and two widely different distome Trematode cercariae, one in the haemocoel and one in the mid-intestine, of both nymphs and adults.

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PLATE I

Figure A.—A nymph in the early ultimate instar, with lateral view of the median gill.

Figures B, C, and D.—Successive changes in the compound eye during the ultimate instar.

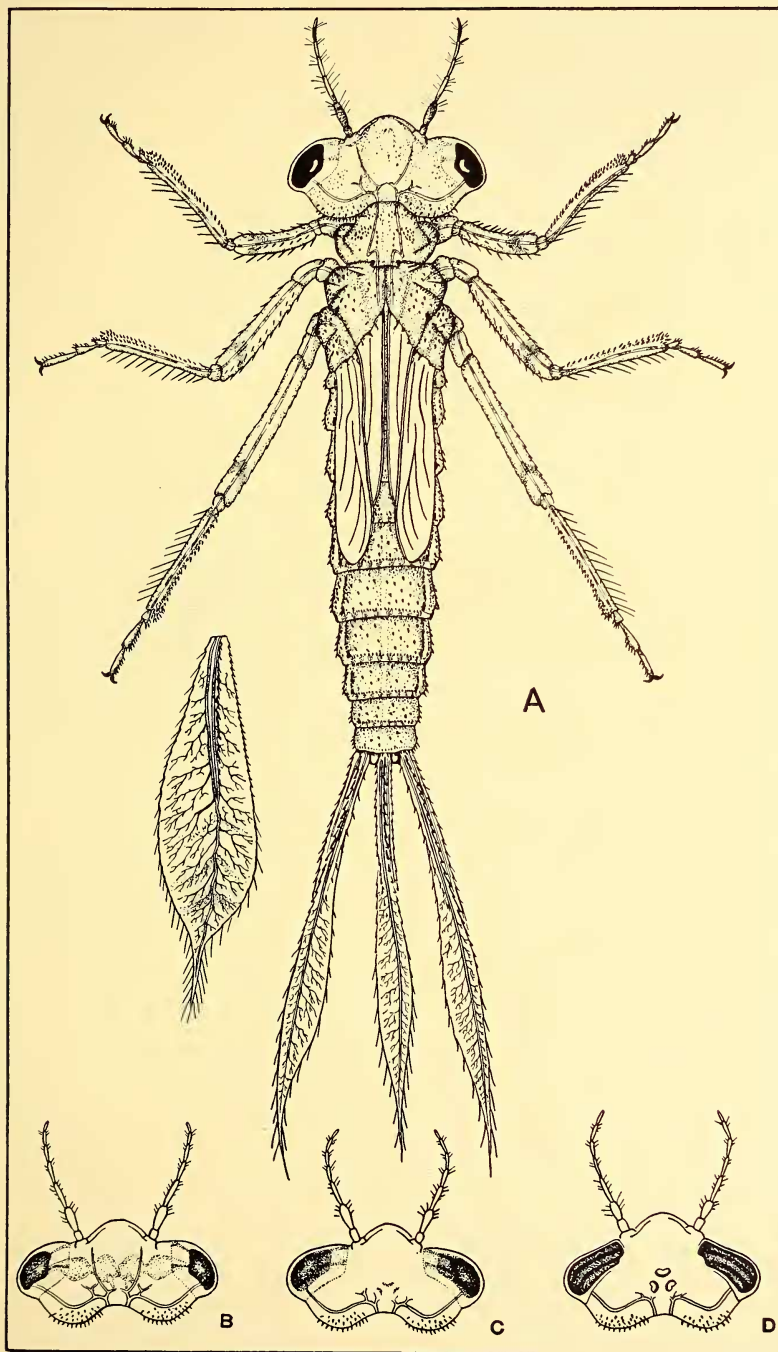


PLATE II

Figures A–E.—Green protozoan, drawn from living material.

A.—Typical sessile form.

B1.—Division by fission.

B2.—A late stage in multiple division.

C and D.—Sessile ovoid pre-flagellate forms.

E.—The flagellate.

Figure F, 1–5.—Fluke no. 1. Cercaria from hemocoel of *I. verticalis*. Sketches from living specimens, showing change of shape in locomotion.

Figure G.—Fluke no. 1A. Metacercaria from cyst in *Anax junius*. Drawn from preserved and stained specimen. Ventral view $\times 120$.

Figure H.—Fluke no. 1A. Encysted metacercariae from *Anax junius* imago, $\times 120$, showing formation of tracheated galls.

Figures J, K, L.—Fluke no. 2. Cercariae from lumen of mid-intestine of *I. verticalis*.

Figure J, 1 and 2.—Sketches of dorsal and lateral views of living specimens.

Figure K.—Dorsal view $\times 120$ of extended living specimen.

Figure L.—Ventral view $\times 120$ of mounted and stained specimen.

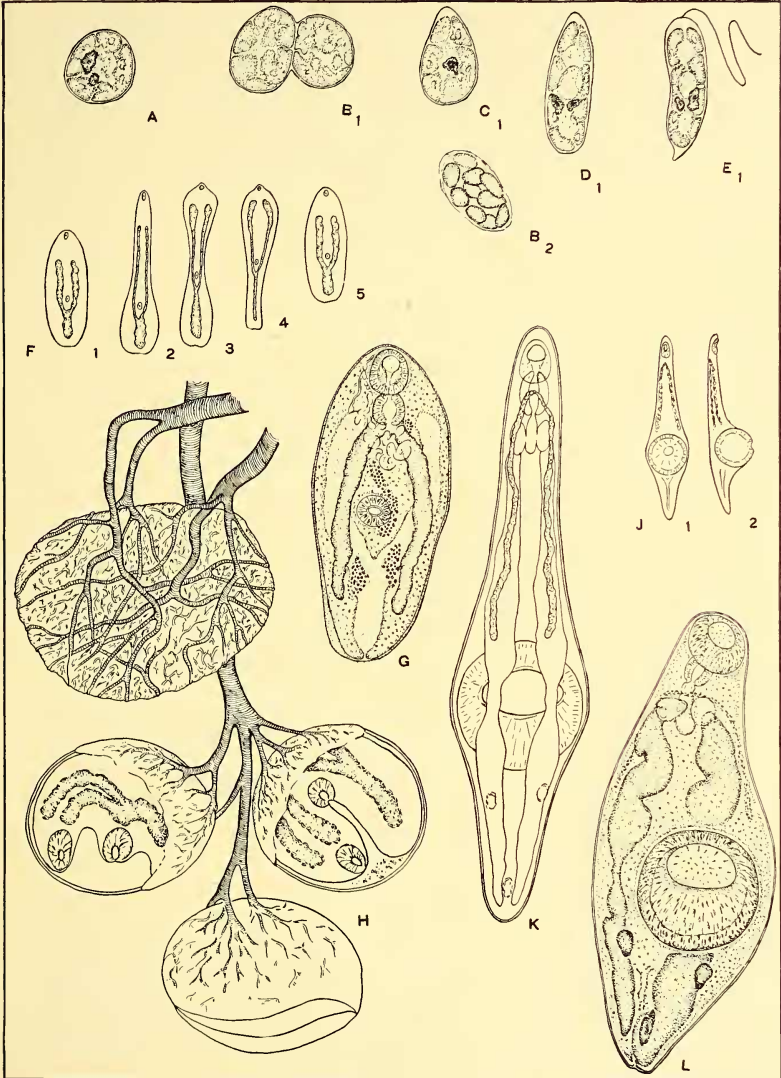


PLATE III

- Figure A.—Side view of a “boat” used for rearing small nymphs. About two-thirds natural size.
- Figure B.—Enamel tray with a group of “boats” floating in the water.
- Figure C.—Glass tumbler in which larger nymphs were kept, until transformation. Note nymph on lower side of bent screen perch.
- Figure D.—Three such rearing cages on a bench in the greenhouse where the work was carried on.
- Figure E.—Aquarium covered by screen cage, used for rearing stock adults, and for oviposition. Note grasses and sedges growing in the water, and sleeve for removing insects, etc. Size of aquarium, 11" × 11" × 11"; cage 12" × 12" × 24".

